

# Statistical Properties of a Two-Stage Model of Carcinogenesis

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Some of the statistical properties of a simple two-stage model of carcinogenesis are explored. The implications of additive treatment effects versus independent treatment effects on the shape of the dose-response curve are considered. Response that is low-dose linear results in the cases where the mutation rates are affected by dose or in the cases where treatment changes the birth rate/death rate of initiated cells in an additive fashion. Independent treatment effects lead to non-low-dose linear response when the survival of initiated cells is affected by treatment. A computer simulation experiment was performed that examined the ability of animal carcinogenesis data to differentiate between various forms of this simple two-stage model. It is shown that animal carcinogenicity experiments do not contain enough data to adequately describe the difference between these two types of effects.

## Introduction

Dose-response models have been used for many years in the quantitative analysis of animal carcinogenesis data. A review of some of the earliest models is given by Krewski and Brown (1). Recent developments in the areas of cell biology, biochemistry, molecular biology, etc., have led to more complicated mechanistic models of carcinogenesis. These mechanistic models have several advantages over simpler probability models or tolerance distribution models. As these models utilize knowledge of the carcinogenic process, they aid in developing improved testing procedures and in explaining a broad range of experimental outcomes. Also, these models generally utilize parameters that have some type of mechanistic interpretation (e.g., mutation rates) and information on these parameters may be available from experiments other than the usual long-term chronic bioassay. These models also have their limitations. Many of these mechanistic parameters are difficult to obtain, requiring specialized biochemical procedures that must be developed for each compound. When information from experiments other than the typical carcinogenicity experiment is available, the inclusion of this information into the risk estimation process is difficult, requiring assumptions that may have questionable biological applicability.

One mechanistic model of carcinogenesis is the multistage model (2-4). The original form of this model has been modified to encompass changes in our understanding of the carcinogenic process (3,5,6). An excellent review of the mathematical development of the multistage model of carcinogenesis is given by Whittemore

and Keller (4). In what follows, a very simple two-stage model of carcinogenesis is studied. Two issues will be discussed: the shape of dose-response curves derived from this simple two-stage model and the ability of animal carcinogenicity data to differentiate between the different shapes.

## Clonal Two-Stage Model

The evidence that carcinogenesis is a multistage process is derived from several sources (2,5,7-9). A simple description of this process is given by the following clonal two-stage model. It is believed that in many cases, the first stage of the carcinogenic process is a mutation (10-12). This collection of mutated or initiated cells are allowed to clonally expand by incorporating birth rates and death rates for these cells. Finally, this two-stage model requires a second mutation to transform these initiated cells into malignant cells.

Figure 1 illustrates the compartments of this model and displays the notation we will use for the rates discussed above. The birth and death rates for normal cells are denoted by  $\beta_0$  and  $\delta_0$ , respectively. Let  $\mu_1$  denote the mutation rate of normal cells into initiated cells. The parameters  $\beta_1$  and  $\delta_1$  represent the birth rate and death rate of initiated cells. Finally, let  $\mu_2$  denote the mutation rate of initiated cells into malignant cells. The model assumes all six of these rates are constant with respect to time. For mathematical simplicity, it is also assumed that  $\beta_0 = \delta_0$ , which is consistent with the assumption that the number of normal cells is constant over time and is not affected by treatment level. It should be noted that normal cells still die and divide. The implications of varying the size of the population of normal cells will be discussed in the last section of this paper.

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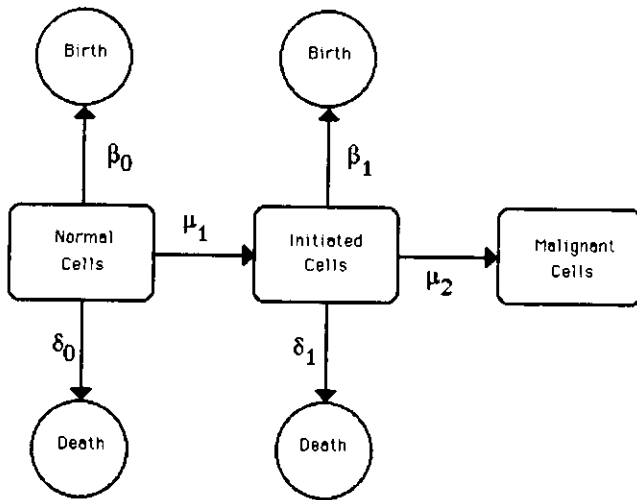


FIGURE 1. A simple clonal two-stage model.

Let  $X$  denote the equilibrium number of normal cells in the tissue being studied, and let  $Z(t;d)$  denote the number of malignant cells at time  $t$  in animals receiving dose  $d$  of the compound being studied. In general, there is a latency period or progression stage from the birth of a malignant cell until tumor detection. This progression could itself be a stochastic or deterministic process, possibly related to treatment. For the arguments presented below, it is only necessary that the progression stage be independent of treatment. Since it is unlikely that various reasonable choices for the form of this progression would have a serious impact on the results, we have chosen the simplest progression model, rapid development of a tumor. If treatment affects the time from malignant cell formation to tumor detection, results other than those presented here could result.

With these assumptions, our objective is to model the probability of at least one malignant cell as a function of both time and dose. For untreated animals, this is given by Moolgavkar (13) as:

$$Pr[Z(t,0) \geq 1] = 1 - \exp \left[ -\mu_0 \mu_1 X \frac{\exp[\beta_1 - \delta_1]t - 1 - (\beta_1 - \delta_1)t}{(\beta_1 - \delta_1)^2} \right] \quad [1]$$

The three parameters  $\mu_0$ ,  $\mu_1$ , and  $X$  enter into the model (Eq. 1) as a product which will be denoted by  $\omega_0$ . It is possible that  $\mu_0$  could be increased and  $\mu_1$  decreased in such a way that the probability expressed by Equation 1 would not change. In mathematical terminology, this means that the parameters  $\mu_0$ ,  $\mu_1$ , and  $X$  are non-identifiable from a single experiment. The only identifiable quantity is their product. Thus, from an animal carcinogenicity experiment, we could estimate  $\omega_0$ , but we could not separate this estimate into its component parts  $\mu_0$ ,  $\mu_1$ , and  $X$  without further information obtained from other sources. Similarly,  $\gamma_0 = \beta_1 - \delta_1$  is identifiable, but  $\beta_1$  and  $\delta_1$  are not. Thus, when fitting animal carcinogenicity data to this model, we can only estimate the two parameters  $\omega_0$  and  $\gamma_0$  and must use

additional data to estimate the full set of five parameters. This presents two additional problems which must be considered when interpreting the results. In many cases, parameters derived from other sources are based upon an educated guess. Second, when one uses information from multiple experiments, care must be taken to include the uncertainty of this information into the model.

## Incorporating Dose Effects

Risk assessment is a problem of assessing treatment effects. There are numerous ways in which dose can enter into a model of this type. The simplest way of incorporating dose into this model is to assume a proportional effect of dose on the two parameters  $\gamma_0$  and  $\omega_0$  in either an independent or an additive manner.

In this situation, the term additive is used to indicate that treatment augments an ongoing process. Thus, if treatment induced a proportional increase in either of the two mutation rates in this model, the single parameter  $\omega_0$ , which will be referred to as the mutation rate, would be expressed using two parameters in the form  $\omega_0 + \omega_1 d$  where  $d$  represents the treatment level. Similarly, if treatment caused a proportional change in either the birth rate or the death rate of initiated cells, the model would be modified to include the form  $\gamma_0 + \gamma_1 d$ . Note that it is not possible to determine whether treatment affects the first mutation or the second when the exposure is over the entire lifetime of the animal. Similarly, if treatment affects the rate of birth and/or death of initiated cells, it is not possible to determine whether that effect is on the birth rate, the death rate or both. This additive clonal two-stage model is given by:

$$Pr[Z(t,d) \geq 1] = 1 - \exp \left[ -(\omega_0 + \omega_1 d) \frac{\exp[\gamma_0 + \gamma_1 d]t - 1 - (\gamma_0 + \gamma_1 d)t}{(\gamma_0 + \gamma_1 d)^2} \right] \quad [2]$$

To simplify the notation later, define  $S[t;d,\omega_0,\omega_1,\gamma_0,\gamma_1] = 1 - Pr[Z(t,d) \geq 1]$ .

Figure 2 illustrates the behavior of this model as a function of both dose and time. The parameter values for this example are  $\omega_0 = 8.55 \times 10^{-7}$ ,  $\omega_1 = 3\omega_0$ ,  $\gamma_0 = 0.041$ , and  $\gamma_1 = 0$ . These parameters were derived from fitting this clonal two-stage model to the historical control data for female Fischer 344 rats (14). Since  $\gamma_1 = 0$ , Figure 2 illustrates a situation in which the treatment only affects the mutation rates. In terminology used by several authors (13,15,16), agents that act in this manner would be labeled as "initiators." Figure 3 illustrates the dose-response curve for this model after 2 years of exposure. Similarly, letting  $\omega_1 = 0$  and  $\gamma_1 = \gamma_0/2$ , the dose-response curve at 2 years is altered as shown in Figure 4. An agent that acts in this manner has been referred to as a "promoter." The major difference between Figure 3 and Figure 4 is in the degree of curvature of the dose-response relationship; Figure 3 more closely agrees with a linear function than does Figure 4.

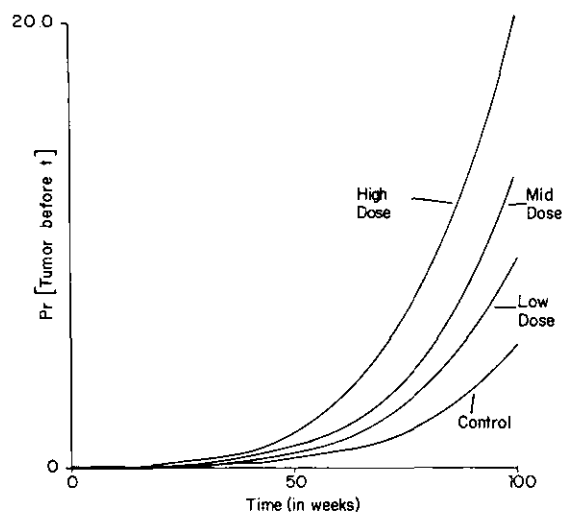


FIGURE 2. Time and dose response for an initiator.

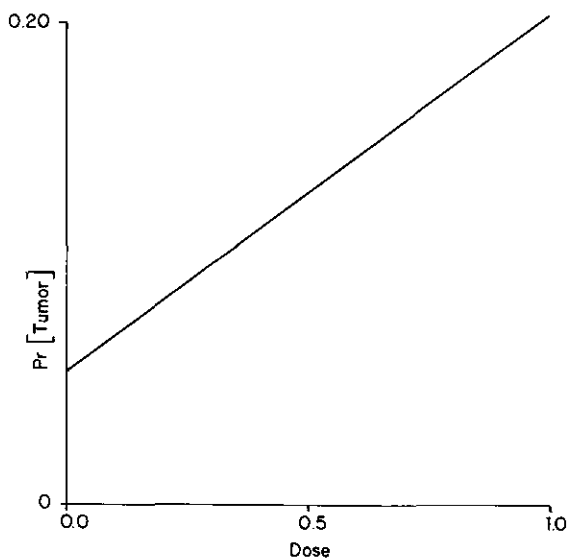


FIGURE 3. Dose response at 104 weeks for an initiator using an additive treatment effect.

A major concern in carcinogenic risk assessment is whether or not a model is low-dose linear. This term is somewhat misused because it is quite easy to show that any continuous function can be approximated by a linear function in a specified small range. What is meant by low-dose linear is that the slope of the dose-response curve at  $d = 0$  is greater than zero. If the slope of the dose-response curve is greater than zero at  $d = 0$ , a small increase in dose will result in a proportional increase in risk. On the other hand, if the slope of the dose-response curve at  $d = 0$  is zero, then a small increase in dose will result in virtually no change in risk. For this reason, dose-response models that are low-dose linear generally estimate smaller acceptable exposures than models which are not low-dose linear.

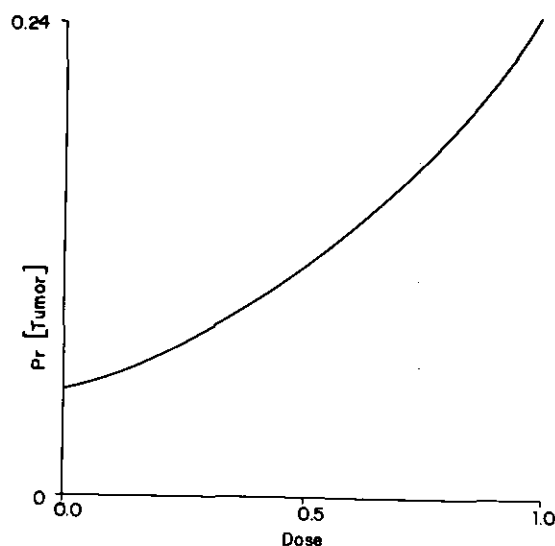


FIGURE 4. Dose response at 104 weeks for a promoter using an additive treatment effect.

Both of the models shown in Figures 3 and 4 are low-dose linear. In fact, it is easy to show that if spontaneous tumors exist, the model given in Equation 2 will be low-dose linear. Thus, additive treatment effects that are proportional to dose will result in low-dose linear models.

Independent treatment effects refer to the situation in which treatment results in an entirely new carcinogenic mechanism or modifies a process that protects against carcinogenesis. Examples of independent effects would include the development of unusual mutations, certain types of cytotoxicity, and increased survival of initiated cells that usually die very rapidly. Figure 5 illustrates one situation for which independent treatment effects might arise. In this example, there are two paths to carcinogenesis; the top path has a positive mutation rate and a positive birth rate for initiated cells, resulting in spontaneous tumors. The bottom path has a low mutation rate and a high death rate for initiated cells, resulting in no spontaneous carcinogenesis. An independent treatment effect would only affect the bottom path in this simple example.

To encompass this model it is necessary to extend the notation to include a second subscript. Thus, let  $\omega_{01}$  denote the mutation parameter in the first (top) path. Similarly define the remaining parameters as  $\gamma_{01}$ ,  $\omega_{02}$ ,  $\omega_{12}$ ,  $\gamma_{02}$ , and  $\gamma_{12}$ , where the second subscript denotes the path to which the parameter applies. Note that since treatment does not affect the top path, it is assumed that  $\omega_{11} = 0$  and  $\gamma_{11} = 0$  and are not included in the list above. Define  $P_1(t) = 1 - S[t|d, \omega_{01}, \omega_{11} = 0, \gamma_{01}, \gamma_{11} = 0]$  (the probability of at least one malignant cell via the first path) and  $P_2(t|d) = 1 - S[t|d, \omega_{02}, \omega_{12}, \gamma_{02}, \gamma_{12}]$  (the probability of at least one malignant cell via the second path). Since it is assumed that all spontaneous mutations occur via the first path,  $P_1(t) > 0$  and that  $P_2(t|d = 0) = 0$ . The probability of at least one malignant

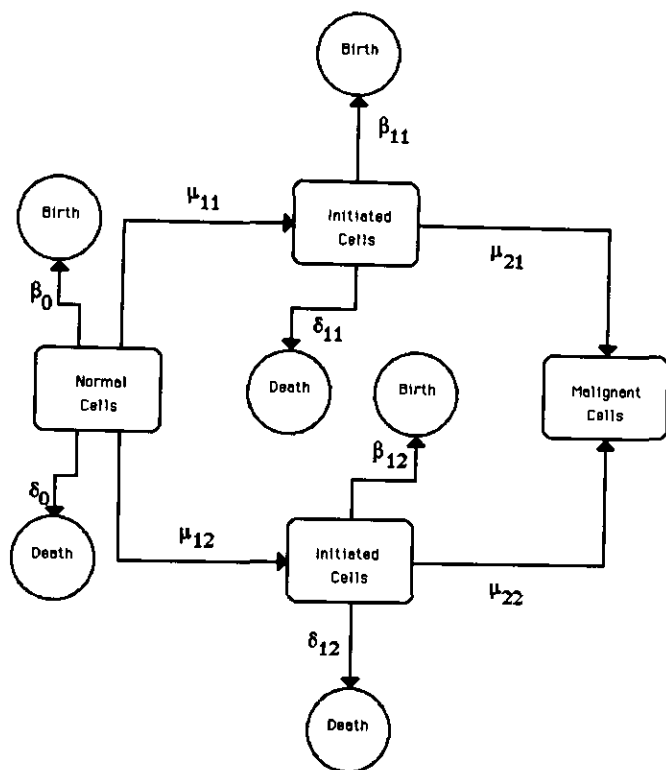


FIGURE 5. A multipath clonal two-stage model.

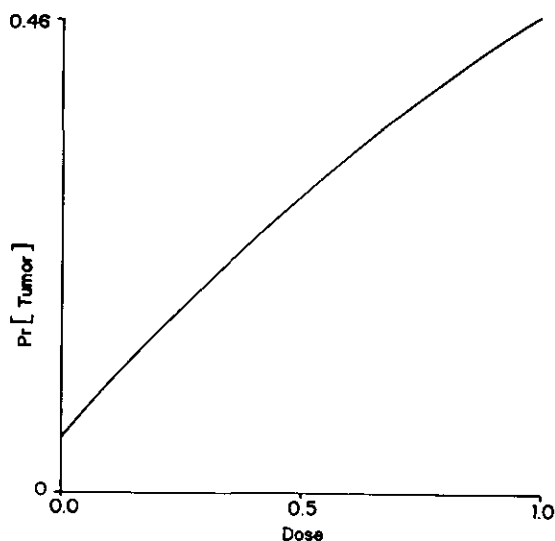


FIGURE 6. Dose response at 104 weeks for an initiator using an independent treatment effect.

nant cell via either path is given by  $P(t|d) = 1 - (1 - P_1(t))(1 - P_2(t|d))$ .

Figure 6 illustrates this model at 104 weeks using parameter values given by  $\omega_{01} = 1.15 \times 10^{-2}$ ,  $\gamma_{01} = 4.72$ ,  $\omega_{02} = 0.001$ ,  $\omega_{12} = 3.0$ ,  $\gamma_{02} = 4.0$ , and  $\gamma_{12} = 0$ . The parameters  $\omega_{01}$  and  $\gamma_{01}$  are derived from fitting the two-stage model to the historical control data

(14) on the incidence of adrenal pheochromocytomas in female Fischer 344 rats. In the terminology presented above, this would represent an initiation effect of treatment. As is obvious by this graph, this type of effect results in a low-dose linear model. In Figure 7,  $\omega_{12}$  is set to 0 and  $\gamma_{12} = 15$ , a very strong promotion effect. It is obvious from this plot that an independent promotion effect results in models which are not low-dose linear and have a slope of zero at dose = 0.

In general, when the background tumor rate is small or zero, treatment will affect these models in the same manner as independent treatment effects. That is, initiators will appear to be low-dose linear and promoters will not.

In summary, we see that in most cases, treatment effects in this two-stage model will exhibit low-dose linear behavior. This includes the additive treatment effects described above and any proportional treatment effects on the mutation rate. The only case for which this model displayed nonlinear low-dose behavior was for independent promotional effects.

## Utility of Carcinogenesis Data for Determining Mechanism

The results of animal carcinogenicity experiments are used to assess the risk from exposure to environmental agents (17). To illustrate additional problems with the use of this model, computer simulation of animal carcinogenicity data can be used. The technical details concerning the way in which animal carcinogenicity experiments are simulated has been presented elsewhere (18). Effectively, the procedure is as follows. First, a design is chosen that determines the doses and the number of animals per dose. The simulations that follow use a three-dose design with 50 animals per dose and doses

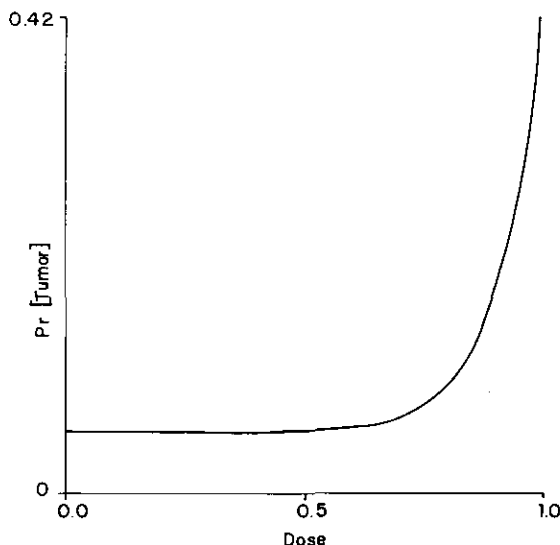


FIGURE 7. Dose response at 104 weeks for a promoter using an independent treatment effect.

of 0.0, 0.25, 0.50, and 1.0. All animals alive at the end of 2 years are sacrificed. Values are chosen for the parameters in the clonal two-stage model and for a model of general survival (19). These parameters are used to determine the probability of survival and the probability of one or more malignant cells for each time and for each dose group based upon the equations presented earlier. Random numbers are used to determine random death times and to decide if a malignancy is present at that death time or not. After repeating this procedure for 200 animals (50 per group for 4 groups), an animal carcinogenicity experiment has been simulated with an underlying time-dose-response relationship determined by the clonal two-stage model and the survival model.

It has been suggested that the clonal two-stage model applied to animal carcinogenesis data could be used to make suggestions about the mechanism of action of some carcinogens (13,15,16). Judging from Figures 3 and 4, it seems unlikely that we would be able to differentiate between additive initiation and promotion effects when background response exists. Simulation experiments provide a means to determine if the ability to differentiate between various mechanisms is possible. Basically, data are generated from either a promotion-only model or an initiation-only model. When fitting this model to simulated carcinogenicity data, it is possible to get small promotional effects in the experimental range that have no impact in the low-dose range and vice-versa for initiation effects. To allow for this, the definition of an initiator was modified to include all cases where  $\omega_1$  dominates the estimate of added risk in the low-dose range even though  $\gamma_1$  may be positive (see Appendix). Similarly, the definition of a promoter was modified to allow for the added risk estimate to be dominated by the estimate of  $\gamma_1$  in the low-dose range. Two other outcomes are possible in the simulations; no significantly increased dose response and significantly increased dose response, which results in both  $\omega_1$  and  $\gamma_1$  affecting the estimated low-dose risk.

Table 1 shows the percentage of times in 1000 simulated data sets that an initiator is correctly classified. For this table, data were generated assuming an additive treatment effect on  $\omega_0$  and assuming  $\gamma_1 = 0$ . As the slope of the dose-response curve increases (column 1), our ability to correctly classify initiators improves. However, it is clear from Table 1 that animal carcinogenesis data does not provide enough information to correctly classify initiators, with the possible exception of very steep dose-response curves ( $\omega_1/\omega_0 = 3$ ). When data are generated using an additive promotion effect,

**Table 1. Classification of simulation outcomes for cases where the chemical is an initiator and treatment effects are additive.**

$\omega_1/\omega_0$	% Initiators	% Promoters	% Both	% Considered
0	29.7	16.2	54.1	3.7
0.5	31.5	26.2	42.3	13.0
1	26.7	33.3	40.0	36.3
2	40.0	26.7	33.3	82.1
3	56.8	20.7	22.5	98.0

a table similar to Table 1 results, with promotion effects being correctly classified about 60% of the time for the steepest dose-response model considered. Thus, we find that when treatment effects are additive, we cannot differentiate between initiation effects and promotion effects.

As mentioned earlier, one advantage of mechanistic models is that some parameters may be available from sources other than the cancer bioassay. Assuming these parameters are for untreated animals, we see from Tables 2 and 3 that knowledge of one of the spontaneous model parameters does not improve our ability to differentiate between initiation effects and promotion effects.

When the background tumor rate is greater than zero and treatment effects are independent, the ability to differentiate between initiation effects and promotion effects is not improved. In Table 4, we have generated experiments for which there is only a promotional effect ( $\gamma_{12} > 0$ ,  $\omega_{12} = 0$ ) using the independent treatment effect model described above. It is clear that even when the promotion effect is very strong, there is a chance that a small initiation effect will dominate the low-dose risk estimates. Again, as is illustrated by Table 5, knowledge of one of the spontaneous parameters does not noticeably improve the ability of animal carcinogenesis data to differentiate between promoters and initiators.

The examples presented above are for the cases

**Table 2. Classification of simulation outcomes for cases where the chemical is an initiator, treatment effects are additive, and  $\omega_0$  is known without error.**

$\omega_1/\omega_0$	% Initiators	% Promoters	% Both	% Considered
0	7.7	30.8	61.5	2.6
0.5	15.8	31.5	52.7	12.7
1	23.2	32.7	44.1	36.7
2	41.9	26.9	31.2	82.3
3	56.4	20.9	22.7	98.3

**Table 3. Classification of simulation outcomes for cases where the chemical is an initiator, treatment effects are additive, and  $\gamma_0$  is known without error.**

$\omega_1/\omega_0$	% Initiators	% Promoters	% Both	% Considered
0	11.1	25.9	63.0	2.7
0.5	19.2	29.6	51.2	12.5
1	26.3	33.1	40.6	35.7
2	41.5	26.8	31.7	82.2
3	56.6	20.7	22.7	98.0

**Table 4. Classification of simulation outcomes for cases where the chemical is a promoter and treatment effects are independent.**

$\gamma_{12}$	% Initiators	% Promoters	% Both	% Considered
0	82.4	11.8	5.8	1.7
10	77.8	16.7	5.5	1.8
12	77.5	22.5	0.0	4.0
14	51.8	47.0	1.2	32.8
17	37.4	60.0	2.6	99.9

Table 5. Classification of simulation outcomes for cases where the chemical is a promoter, treatment effects are independent, and  $\omega_{01}$  is known without error.

$\gamma_{12}$	% Initiators	% Promoters	% Both	% Considered
0	88.9	11.1	0.0	1.8
10	78.9	21.1	0.0	1.9
12	68.6	28.6	2.8	3.5
14	48.9	50.2	0.9	32.7
17	35.6	62.6	1.8	100.0

where there exists a background tumor response. For the case of very small or zero background response, the ability to differentiate between initiators and promoters improves considerably, approaching perfection for steep dose-response curves. Note also that we are discussing low-dose initiation versus low-dose promotion. Changing the definition of "low dose" and modifying the definitions of "initiators" and "promoters" could obviously alter the results of this analysis.

Finally, to show how random chance can lead to an incorrect interpretation of bioassay results, consider the estimated model shown in Figure 8. Curves with this shape occurred in 0.5 to 2% of the simulations. If one were to estimate this model from animal carcinogenesis data, one would conclude that the chemical is highly mutagenic at low doses and highly cytotoxic to initiated cells at high doses. However, this is simply the realization of a random event from a model which in truth looks nothing like this model. Thus, one should be very careful in interpreting the results of curve fitting when using a model as flexible as this one.

## Summary

This manuscript has discussed two topics concerning the use of mechanistic models in carcinogenic risk as-

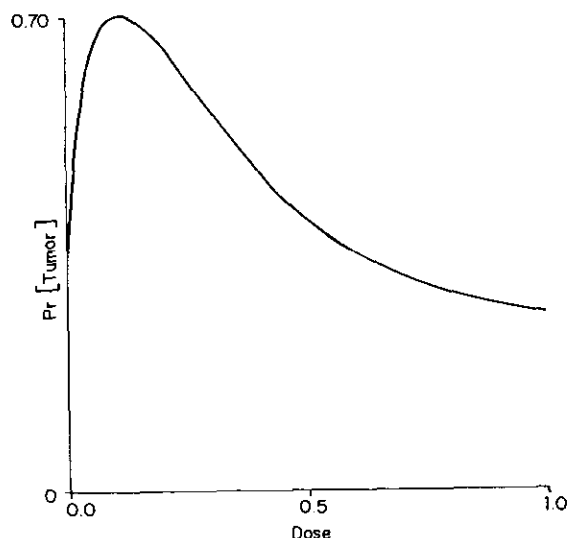


FIGURE 8. Dose-response at 104 weeks for a sample data set that suggests the compound is an initiator and toxic to initiated cells.

essment. The first problem concerns the incorporation of treatment effects into the model. It was shown that if treatment enters into the model in an additive fashion, then the model will behave in a low-dose linear fashion. If, on the other hand, background response is near zero or treatment can be incorporated in an independent fashion, it may be possible to have nonlinear low-dose risk. The second issue concerns the ability of animal carcinogenesis data to differentiate between alternative mechanisms of carcinogenesis. In this case it was shown that it is not possible to differentiate between mechanisms for a very simple two-stage model.

The two-stage model considered in this study did not allow for changes in the size of the susceptible normal population. Hormonal changes in the animals could result in age-related changes in the size of the susceptible population (20,21). It is also possible that the treatment could be cytotoxic, reducing the size of the susceptible population and/or increasing the mitotic rate in these cells. It is possible that these mechanisms could play a significant role in the formation of tumors. In general it is believed these mechanisms would result in dose-response relationships that will not be low-dose linear. As is the case above, this will depend upon whether they are proportional effects and whether these effects are independent or additive.

Despite the problems mentioned above, mechanistic models serve an important role in carcinogenic risk assessment. When one is extrapolating beyond the range of the data, one would like to use a model that contains the largest amount of information available on the process being modeled. In addition, these models are very useful for developing alternative designs and novel experiments to address broader issues in cancer risk assessment. They also provide a conceptual framework in which to think about experimental results and help to combine information from a variety of experiments. However, one should be careful not to overinterpret the parameters arrived at by fitting mechanistic models to animal data.

## APPENDIX

As mentioned in the text, the definitions of initiators and promoters were modified for dealing with model estimates from the simulations. For the purposes of risk assessment it is too strict to require that estimated parameters be identically zero before a classification can be made. Instead, since low-dose risk assessment is the goal of our analysis, the definition was modified so that a simulated response was labeled as an initiator if the dose-related mutation parameter ( $\omega_1$ ) dominated the low-dose risk estimate. This was done by calculating the four values  $S_d = S(104|\epsilon, \omega_0, \omega_1, \gamma_0, \gamma_1)$ ,  $S_p = S(104|\epsilon, \omega_0, \omega_1 = 0, \gamma_0, \gamma_1)$ ,  $S_i = S(104|\epsilon, \omega_0, \omega_1, \gamma_0, \gamma_1 = 0)$ ,  $S_0 = S(104|\epsilon, \omega_0, \omega_1 = 0, \gamma_0, \gamma_1 = 0)$  for  $\epsilon = 10^{-5}$ . The value of  $S_0 - S_d$  estimates the added risk of tumor from exposure to a dose  $\epsilon$  of the simulated chemical. Since  $S_p$  uses  $\omega_1 = 0$ , then  $S_0 - S_p$  estimates the added risk from exposure to a dose  $\epsilon$  when the dose-

related mutation parameter is set to zero (without estimating the remaining parameters again). If  $S_0 - S_p$  is close in value to  $S_0 - S_d$ , then one could conclude that the promotion effect is very strong at low doses. Similarly,  $S_0 - S_i$  estimates the added risk for a dose of  $\epsilon$  using only the initiation effect. A simulated effect was considered to be a low dose initiation effect if  $(S_0 - S_i)/(S_0 - S_d) - (S_0 - S_p)/(S_0 - S_d) > 0.10$ . That is, the relative initiation effect is 10% larger than the relative promotion effect. Similarly, if this quantity was less than  $-0.10$ , the simulated effect was assumed to be a promotion effect. Since, for very low doses, small mutation effects could dominate the added risk estimate, we also considered  $\epsilon = 0.01$ . The results in Tables 1-3 are for  $\epsilon = 10^{-5}$  and those in Tables 4 and 5 are for  $\epsilon = 0.01$ .

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